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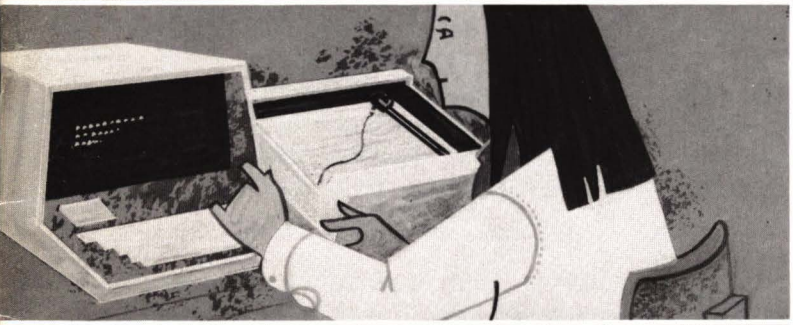

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EVALUATION OF THE SPRAY NOZZLE TYPE AND CONFIGURATION IN THE APPLICATION OF SPORE SUSPENSIONS OF THE FUSIFORM RUST FUNGUS TO SOUTHERN PINES

D.B. Drummond, S.D. Hubbard, and J.W. Barry[1]

ABSTRACT

A comparative evaluation of nozzle types and configurations used to apply a Cronartium quercuum f. sp. fusiforme spore suspension to seedlings of Pinus taeda L. was made during March 1978, at the USDA Forest Service Resistance Screening Center at Bent Creek, North Carolina. Commercially manufactured nozzles increased the numbers and the volume of spore suspension droplets applied to seedlings. Application appeared more consistent when compared to previously used custom-made nozzles. Use of the commercially manufactured nozzles should provide for more reliable results when evaluating the resistance of various seed lots to the fusiform rust fungus.

INTRODUCTION

The USDA Forest Service Resistance Screening Center, Bent Creek, North Carolina, annually tests approximately 500 seed lots for resistance to Cronartium quercuum (Berk.) Miyabe ex Shirai f. sp. fusiforme (Cumm.). This fungus causes fusiform rust, now considered to be the most destructive disease of southern pines.

Tests to determine relative disease resistance of loblolly pine seedlots (Pinus taeda L.) to fusiform rust require over a year to complete. Therefore, each test represents a substantial investment. The screening process involves exposing seedlings from several seed lots to inoculum of the fusiform rust fungus. The resistance of individuals from different pine seed lots can be compared only if all seedlings are inoculated under similar conditions. It is important that procedures are consistent so that the representatives of each seedlot receive the same amount of inoculum. Each seedling screened should, after spraying, retain the same number and size of spore suspension droplets.

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The Resistance Screening Center uses an automated system to inoculate seedlings. A conveyor belt moves seedlings at a constant speed through a spore suspension spray and into an incubation chamber. The density of spores in the suspension and the flow of inoculum are carefully controlled. Two flanking nozzles, custom-made in the Iowa State University instrument shop[1], have been used to spray the inoculum suspension (fig. 1).

The objective of this evaluation was to determine which of two nozzle types, the original custom-made Ames nozzles or recently acquired Spraying Systems Co. nozzles (1/4 J + Su1), and which of two nozzle configurations, two flanking and one overhead, would provide the most consistent application of the suspension on seedlings.

METHODS

On March 15-16, 1978, Rhodamine B extra S dye was mixed and applied with a spore suspension containing 50,000 basidiospores/ml to loblolly pine seedlings in seven trials. The trials were as follows:

1. An inoculation system, consisting of two Ames nozzles with orifice diameters of .059 inches, was used to apply the test material. Nozzles located on either side of and approximately 18 inches above a conveyor belt were turned approximately 40° from the straight-down position toward the center of the conveyor belt (fig. 2). The spore suspension reservoir was pressurized and the seedling foliage was wetted prior to application of the spore suspension.

2. The same as in Trial 1 except the inoculum was applied to dry foliage. This represents the inoculation procedure used operationally at the Resistance Screening Center from 1973-1978. In all remaining trials the seedling foliage was dry.

3. Trial 3 consisted of two Spraying Systems nozzles (1/4 J + Su1). The nozzle configuration was the same as with the Ames nozzles in Trials 1 and 2 above. The spore suspension was siphoned from a nonpressurized reservoir.

4. Trial 4 was set up with one Spraying Systems nozzle located directly over and approximately 2 feet above the conveyor belt. It was pointed straight down. A nonpressurized reservoir system was used.

5. Trial 5 was the same as Trial 4; however the nozzle was canted approximately 10° toward the oncoming seedlings.

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1 Designated as the Ames nozzles throughout this report.

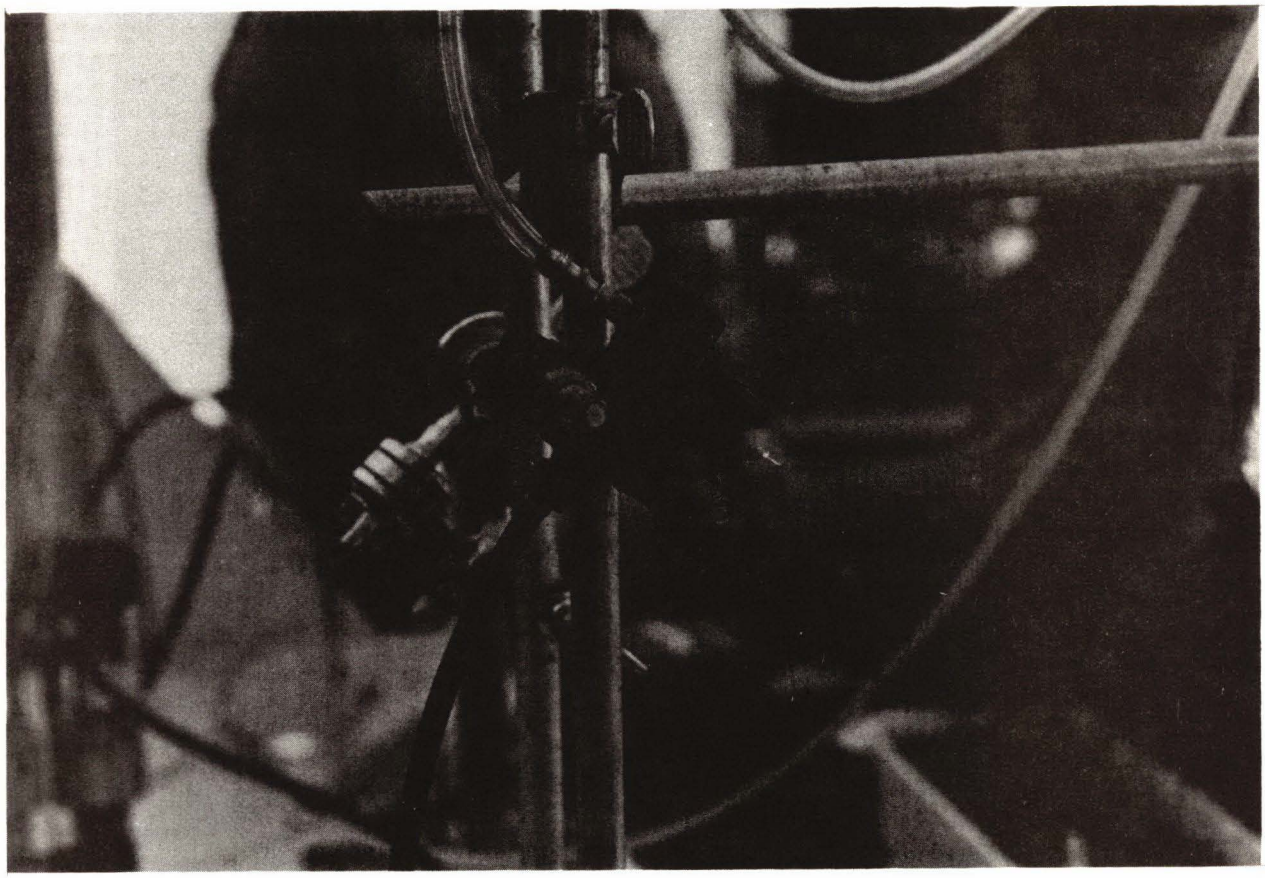

Figure 1. Closeup of one of the Ames nozzles used at the Forest Service Resistance Screening Center prior to this test. Bent Creek, NC. 1978.

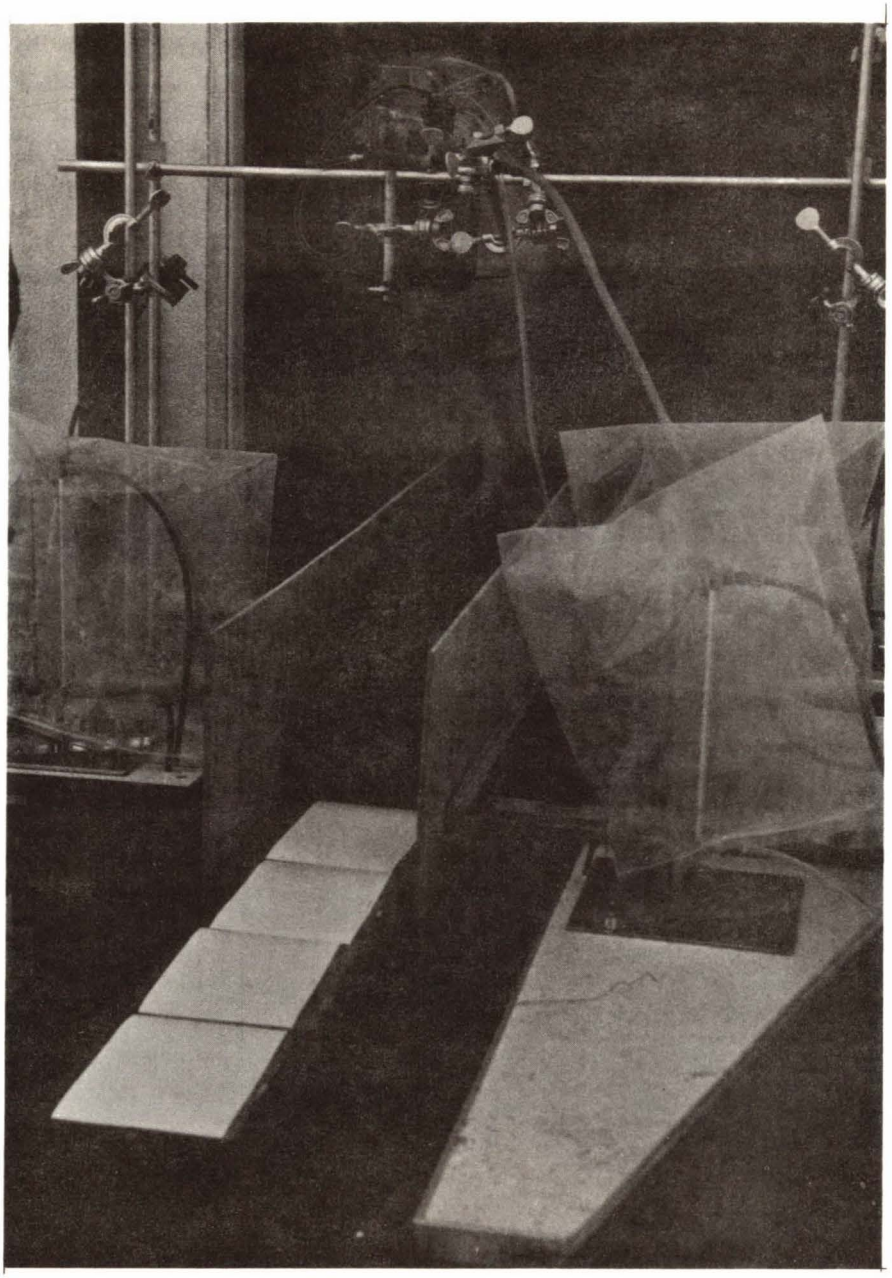

Figure 2. The Ames nozzle assembly with seedlings and Kromekote deposit cards on the conveyor belt moving past the nozzles. Flow meters, controlling the application rate are covered with plastic bags to prevent the dyed spore suspension from being deposited on them. Bent Creek, NC. 1978.

6. Trial 6 duplicated Trial 3 except the spore suspension reservoir was pressurized. The nozzle angle might have differed slightly as a result of take-down and set-up procedures.

7. This trial was the same as Trial 6 with the exception of a sticker added (10 percent by volume) to the spore suspension. This was added in an attempt to increase droplet retention on the seedlings.

Trays of seedlings were placed on a conveyor belt which moved past the nozzles at a rate of 6 feet per minute for those trials with two flanking nozzles (1, 2, 3, 6, 7) and 3 feet per minute for those trials with one overhead nozzle (4, 5). Nozzle flowrate was calibrated to maintain a constant spray discharge of 15 ml per minute per nozzle for all treatments. In this way, about 10 ml per tray of seedlings was discharged regardless of the number of nozzles or their configuration. Trials are summarized in table 1.

Kromekote spray deposit cards were exposed to the spray between seedling trays during each trial. These cards were used to characterize spray deposition using a Quantimet--an image analyzer used to count and size droplet stains. The Quantimet data were then processed with the Automatic Spot Counting and Sizing program (ASCAS) (Young et al. 1977). The ASCAS computer program calculates the droplet diameter from the stain diameter and estimates the volume per unit area when the number of droplets per unit area and droplet sizes are given. The Quantimet and ASCAS procedures provided information on droplet size, droplet number, and spray volume deposited on the Kromekote cards.

Three trays of 20 seedlings each were sprayed in each trial. Visual examination of dyed droplets of inoculum were made. In addition, the red droplets stains were allowed to dry on the seedling foliage and the following data taken:

1. The number and length of mature and immature needles were determined on one seedling from each treatment (seven trees). The length of the succulent stem on each seedling was measured and recorded.

2. Drop stains were counted on the 1st cm from the stem on: one cotyledon; two mature; and three immature needles of 15 seedlings from each of Trials 2, 3, and 5 (45 trees). These three trials represent the three nozzle configurations used in the seven trials and compare the most viable alternatives based upon visual examinations. Evaluation of fusiform rust resistance is based on disease symptoms produced on the stem. The 1st cm from the stem was considered important because infections occurring beyond this zone rarely result in an invasion of the stem by the pathogen (Powers 1968).

Table 1. Inoculation trial conditions, March 15-16, 1978.

Trial	Nozzle Type	Nozzle Angle	Pressure (psi) to Nozzles
1&2	2 Ames nozzles[1]	40°	20
3	2 Spraying Systems[2]	40°	26-28
4	1 Spraying Systems	down	38
5	1 Spraying Systems	10° forward	38
6	2 Spraying Systems	40°	28
7	2 Spraying Systems	40°	28

1 Nozzles were custom-made and designed at Iowa State University, Ames, Iowa.

2 Available from Spraying Systems Co.; nozzle 1/4J+Su1.

3. Drop stains were counted on 1 cm of the stem of 15 seedlings each from Trials 2, 3, and 5.

4. Three seedlings were selected for drop stain diameter measurement from each trial (21 trees). Drop stain diameters were measured on the 1st cm of the stem, one cotyledon, one mature needle and three immature needles. The stain size is proportional to the size of the original or aerodynamic droplet. Stains produced by water base sprays on coniferous foliage are approximately two times the droplet diameter (Barry and Ekblad 1978). Stain diameter was measured manually using a microscope equipped with an ocular micrometer.

5. Diameters of all droplet stains were measured on the total length of one mature and one immature needle from two seedlings for each trial (14 trees).

RESULTS

Visual examination of seedlings sprayed in Trials 1, 2 and 4 showed that these trials gave inferior results in terms of uniformity in number and size of spore suspension droplets. Visual examination of the remaining trials, indicated that Trials 3, 5, 6, and 7, that is, trials using the Spraying Systems nozzles, produced more droplets and more uniform droplets, than did Trial 2, which used the Ames nozzles (figs. 3 and 4).

Droplet Size and Distribution On Kromekote Cards

The first four columns in table 2 summarize data output from the ASCAS program, and in general characterize the droplet size spectrum on Kromekote cards for each trial. In the seven trials there is a similarity of volume median diameters (VMD's) for similar nozzle configurations. The Ames nozzle spray system used in Trials 1 and 2 had a VMD of 93 μm. The configuration of two commercial nozzles (Trials 3, 6, and 7) had VMD's of 71, 71, and 78 μm. The single commercial nozzle configuration (Trials 4 and 5) had VMD's of 55 and 51 μm. These data indicate that the commercial nozzles produced smaller droplets than the Ames nozzles and one commercial nozzle produced smaller droplets than two commercial nozzles. The fifth column of table 2, indicates that with two commercial nozzles more drops were deposited than with one commercial nozzle which deposited more drops than two Ames nozzles. Pressurization of the two nozzle system did not appear to make a large amount of difference. Also, substantially more droplets were deposited in Trial 7; the trial with the sticker added to the dyed inoculum.

Figure 3. Large irregular shaped droplets of spore suspension deposited by the Ames nozzles. Bent Creek, NC. 1978.

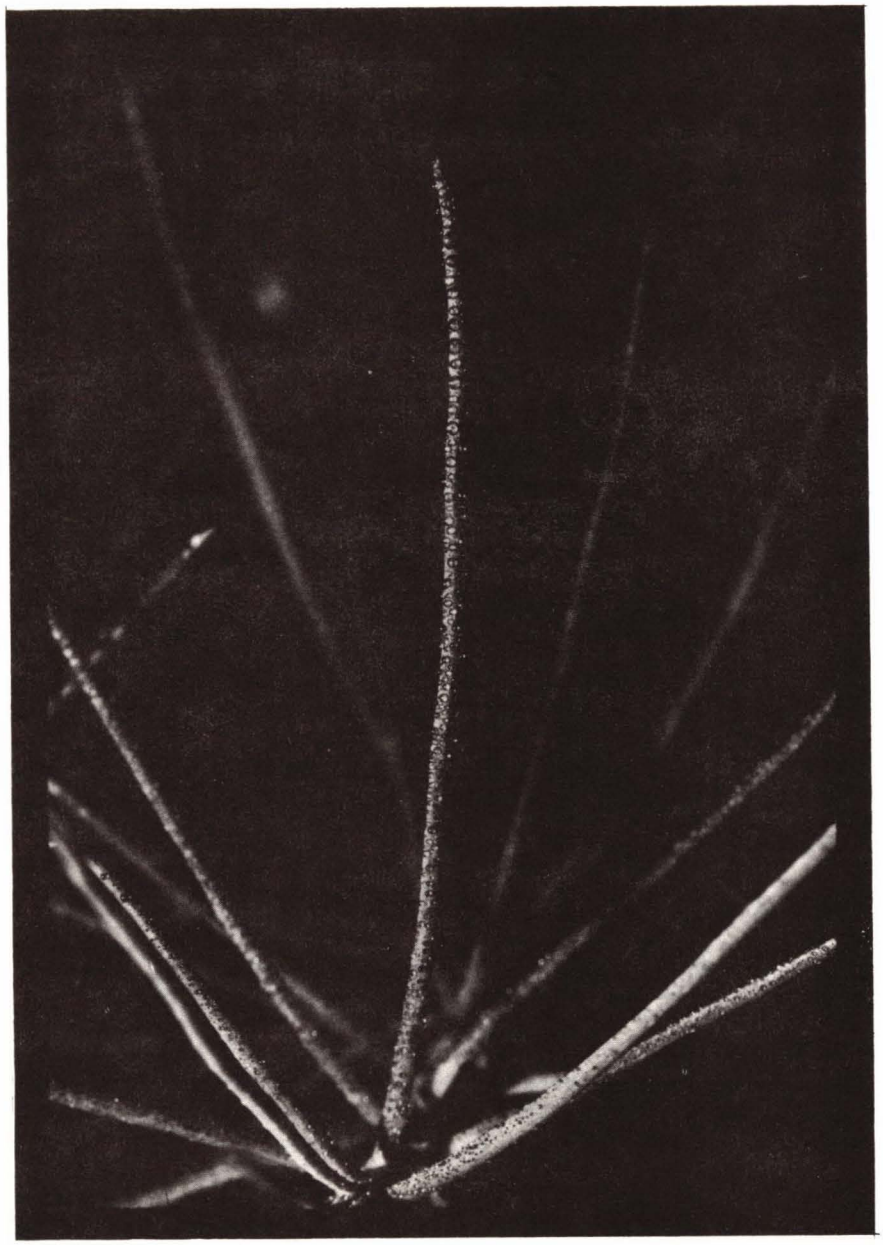

Figure 4. Numerous, small droplets of spore suspension deposited by the commercial nozzles. Bent Creek, NC. 1978.

Table 2. Summary of spray droplet deposition data from Kromekote cards.[1]

Trial	Drop Diameters (μm) Volume median[2]	Volume mean[3]	Number median[4]	Number mean[5]	drops/[6] cm^2	gal/A[7]	grams/[7] M^2
1&2	93	133	33	38	478	0.503	0.4507
3	71	73	26	32	853	0.47	0.4213
4	55	60	19	24	634	0.165	0.1479
5	51	53	15	21	631	0.111	0.0991
6	71	74	22	29	776	0.379	0.3394
7	78	81	25	32	1106	0.74	0.6635

1 Stains were counted and sized by a Quantimet Image Analyzer.

2 Volume Median Diameter (VMD). The drop diameter at which 50% of the volume is in drops smaller and 50% of the volume is in drops larger than the VMD (also referred to as mass median diameter (MMD)).

3 Volume Mean Diameter. The diameter of a drop with the average volume.

4 Number Median Diameter. The drop diameter which divides the total drop population in half with half the drops larger and half the drops smaller.

5 Number Mean Diameter. Average diameter of the drop population, independent of volume.

6 Volumes are calculated from these data.

7 ASCAS program outputs gal/A and grams/meter ^{2}. Convert to more convenient area unit by using appropriate factor.

The VMD and the number of drops per cm^2 on the cards were used by the ASCAS program to estimate the spray volume applied (table 2). Based on the data from Kromekote cards, the volume estimates for one commercial nozzle were much less than for two commercial nozzles. The two commercial nozzles deposited only slightly less volume of inoculum on cards than did the Ames nozzles, but the droplets were smaller than those deposited by the Ames nozzles. One commercial nozzle deposited fewer, smaller droplets, and therefore, less volume than did two commercial nozzles.

Number of Drop Stains on Pine Seedlings

The average number of stains on the 1 cm closest to the stem for each needle type is summarized by trial in table 3. There were more than seven times as many drop stains on needles sprayed with two commercial nozzles as on needles sprayed with Ames nozzles, and five times as many drop stains on needles sprayed with one commercial nozzle as on needles sprayed with Ames nozzles. On the stem tissue, the two commercial nozzles resulted in more drop stains per cm than the other trials, but the magnitude of the difference was not as great as on the needles. On the basis of the number of droplets alone, the double nozzle configuration using commercial nozzles (Trial 3) provided substantially greater numbers of droplets per unit length of needle than did the other two trials.

Droplet Size and Distribution on Pine Seedlings

The number of drops, VMD, and volume of dyed spray, by needle type, deposited on 1 cm of needle length and deposition on 1 cm of stem tissue were determined for three trees from each trial and are presented in table 4. Cotyledons and mature needle types retained greater volumes per cm than the other needle types. The VMD for the needles are generally smaller than those for Kromekote cards exposed in the same trial. The volume per cm of needle length for the various needle types and the three treatments were subjected to an analysis of variance. Data for the stems were not included in the analysis. The results of the analysis of variance were used to determine the upper and lower confidence limits (as defined by Goldstein 1965) and are shown for all needle types combined in table 4.

Trials 2 and 3, and Trials 3 and 5 were significantly different ($P < .05$). Volumes deposited in Trials 2 and 5 were not significantly different from each other. Thus the two commercial-nozzle configuration resulted in greater volumes of spray deposited on the needle surface than did the two Ames nozzles or the one commercial-nozzle configuration.

Table 3. The average number of drop stains on the first cm of one cotyledon, two mature, and three immature needles for 15 trees selected from each treatment.

Treatment	Observations	Cotyledon		Mature				Immature						All Needles	
				middle		upper		largest		random		smallest			
		x	SE	x	SE	x	SE	x	SE	x	SE	x	SE	x	SE
2 Ames nozzles	15	249	40	144	19	196	16	136	21	199	31	43	6	161	10
2 commercial nozzles	14	1375	138	1012	102	1398	137	1313	144	1196	102	744	76	1173	49
1 commercial nozzle	15	1302	202	831	69	848	98	1130	127	691	118	406	57	868	50

The average number of drop stains on 1 cm of succulent stem tissue for 15 loblolly pine seedlings

		x	SE
2 Ames nozzles	15	1157	92
2 commercial nozzles	15	1494	82
1 commercial nozzle	14	1173	163

Table 4. Overall results of number of drops, VMD, and volume of spray deposited on the various needle types and the stem by treatment as determined by ASCAS.

Needle Position		Trial 2	Trial 3	Trial 5
Cotyledon Needles	drops/cm*	894	1364	1050
	VMD[2]**	26.5	37.2	21.9
	mg/cm [x10^{-4}]*	11.8	40.5	11.8
Mature Needles	drops/cm	515	753	799
	VMD	29.7	39.4	13.4
	mg/cm [x10^{-4}]	8.4	19.4	3.2
Largest Immature Needles	drops/cm	426	769	754
	VMD	27.8	16.0	16.0
	mg/cm [x10^{-4}]	3.3	6.0	5.2
Random Immature Needles	drops/cm	149	445	423
	VMD	17.3	10.6	17.2
	mg/cm [x10^{-4}]	1.6	0.9	2.3
Smallest Immature Needles	drops/cm	355	132	240
	VMD	17.2	15.3	10.5
	mg/cm [x10^{-4}]	0.6	0.3	0.5
All Needle Positions	mean mg/cm [x10^{-4}]	5.14^{a1}	13.41^{b}	4.61^{a}
	95% Confidence Limits			
	Lower Limit	0.44	8.70	0.09
	Upper Limit	9.85	18.11	9.32
Stem Tissue	drop/cm	977	1827	1969
	VMD	27.7	18.4	11.6
	mg/cm [x10^{-4}]	10.9	22.1	4.8

* Droplet determinations were made by counting and sizing the drop stains on the first cm from the stem on one cotyledon, two mature needles from each of 3 trees per treatment.

** VMD and volume for the first cm of the needle were calculated using the ASCAS computer system (Young et al. 1977). Data placed in the system were derived by measuring the size of all drop stains on the first cm from the stem on one cotyledon and one mature needle, 3 trees per treatment (table 2).

1 For all needle types, mean mg/cm^2 values followed by the same letter are not statistically different from each other at the 5 percent level.

Table 5. Number and length of all needles on a representative seedling for each treatment, cotyledons were included as mature needles.

Trial	stem length cm	Mature Needles No.	Mature Needles avg. length cm	Mature Needles SE	Immature Needles No.	Immature Needles avg. length cm	Immature Needles SE
2	2.0	41	2.41	.065	26	1.33	.147
3	3.0	44	2.78	.077	32	1.42	.150
5	2.7	45	2.98	.070	32	1.43	.161

Estimating Total Volume of Spray Deposited per Seedling

If the total volume deposited per seedling is known, an estimate of the number of spores deposited per seedling can be made. This can be accomplished by (1) using the concentration of spores per ml in the original spore suspension and (2) regression formulas that relate the number of droplet stains per cm of needle length as determined by counting the droplets on a large number of seedlings, to the volume per cm determined by sizing a proportion of those droplets and using ASCAS to calculate volume. The data from this evaluation were insufficient to allow the confident use of such regressions, so none are presented in this report. However, the procedure outlined in the following paragraphs can be used when more complete data are available. Manually measuring droplet diameters is tedious, time consuming and subject to human error. A new generation of image analyzers soon may be available to automatically count and size droplets on needles, making it possible to increase the sample size. This is not to suggest that manual measuring is less accurate than by use of image analyzers. The following procedure does, however, illustrate a method to estimate total volume of spore suspension applied per seedling. Such estimates would be reliable only if a large number of samples were collected.

The first step is to calculate the average number and size of droplets on the 1st cm by needle type for all treatments. The average estimates of the number of droplets within 1 cm from the stem for both mature and immature needles, can then be used to calculate the volume per cm for each needle type using the appropriate treatment regression.

Using the average number of needles on the representative seedlings from each treatment (table 5), total volume of spray applied to the foliage within 1 cm of and including the stem of the average seedling, can be estimated by multiplying the number of immature and mature needles on the average tree by the appropriate volume per cm for each of the two needle types. These two products, one for mature and one for immature needles, are then added to provide an estimate of total volume of spore suspension applied per tree within 1 cm of the stems.

Assuming an equal distribution of basidiospores in the spore suspension, and uniform distribution of spores in the suspension throughout the flow system, one can then estimate the total number of spores per seedling for a spore suspension containing a known number of spores/ml. Keeping in mind that we lack an accurate estimate of volume per seedling because of the restricted sample size, sample calculations were made and 421; 26,100; and 7,150 spores per seedling were derived for Trials 2, 3, and 5 respectively.

DISCUSSION

The VMD of droplets deposited on the Kromekote cards (table 2) was greater than on seedlings (table 4) which suggests that on the seedlings droplets were coalescing into large drops and falling or being blown off the needles by the force of the spray. Only smaller drops that maintained some individuality and had not increased in size beyond some critical threshold were retained on the needles. Data presented in table 4 for each needle type show that with the two nozzle systems VMD on needles were inconsistent. Because of these inconsistencies in the VMD on needles, the card data probably more accurately reflect the true character of the spray stream. Only on the stem which was much larger in diameter was the droplet size pattern the same as that observed on cards i.e., the largest drops deposited by the two Ames nozzles, the next largest deposited by the two Spraying Systems nozzles, and the smallest with a single Spraying System nozzle. This inconsistency in droplet size on needles would also seem to be related to droplets coalescing upon or before impact. The increase in VMD from a single Spraying Systems nozzle to two Spraying System nozzles detected on the Kromekote cards suggest some combining of droplets in the spray streams prior to deposition when using two nozzles.

Visual observation of droplets on seedlings exposed to both two nozzle systems strongly suggests that more droplets, and more uniform droplets, were retained on needles exposed to the Spray Systems nozzles (figs. 3 and 4). On the basis of these observations, the spray system used operationally at the Resistance Screening Center was modified subsequent to these trials to use the commercial nozzles as in Trial 3.

Data on droplet numbers (table 3) support this observation. Many more droplets were deposited when using the commercial nozzles. Coalescing of droplets with the commercial system may not occur to as great a degree as with the original Ames nozzles, thus fewer droplets would be falling from the needles. With fewer droplets coalescing one also would expect less variation in droplet size.

As a result of increased droplet numbers the total volume was greater using two Spraying System nozzles than with the Ames nozzles. The single Spraying Systems Co. nozzle configuration yielded higher droplet numbers but less volume than the two Ames nozzles. If the two commercial nozzles are used at the same application rates as the Ames nozzles one would expect to have greater numbers of spores applied to the seedlings, and resulting infection levels to increase. This indeed has happened since the two Spraying Systems nozzles have been used operationally at the Resistance Screening Center, and has been demonstrated experimentally (Hubbard and Ryan 1980). The visual observation that the Spraying Systems nozzles yielded more uniform droplets on seedlings has been supported by a decrease in experimental error and an increase in genetic repeatability estimates from

operational tests since the Spraying Systems nozzles have been used (Hubbard 1980). These changes might be due, all or in part, to the increased consistancy of droplets obtained when using the Spraying Systems nozzles.

RECOMMENDATIONS

It is recommended that commercial nozzles be used over handmade (Ames) nozzles and that spray card evaluation be used along with visual observations of dye droplets on seedlings for evaluating spray inoculation systems. Counting dye stains and measuring stain sizes on foliage is not recommended for the sole purpose of evaluating nozzle systems or for checking quality control.

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